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Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV/AIDS patients: Prevalence and antimicrobial susceptibility pattern at the University of Gondar Hospital, North West Ethiopia

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CERTEFICATE

This is to certify that the thesis prepared by Demeke Endalamaw which is entitled with **''Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV patients: Prevalence, antimicrobial susceptibility pattern at the University of Gondar Hospital, North west Ethiopia''** for partial fulfillment of the requirements for the degree of Master of Sciences in Medical Microbiology was carried out under supervision and complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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AIDS	Acquired Immunodeficiency Syndrome
AMPC	Ampicillin class C beta lactamase
ART	Antiretroviral Therapy
ATCC	American Type Culture Collection
CLSI	Clinical Laboratory Standard Institute
CLED	Cystine Lactose Electrolyte Deficient
CTX-M	Cefotaximase-Munich
DDST	Double Disc Synergy Test
MDDST	Modified Double Disc Synergy Test
EPI INFO	Epidemiological Information
ESBL	Extended Spectrum Beta Lactamase
ESBL-PE	Extended Spectrum beta Lactamase producing Enterobacteriaceae
HIV	Human Immunodeficiency Virus
IV	Intravenous
LIA	Lysine Iron Agar
MAC	MacConkey
MDR	Multidrug Resistance
MHA	Muller Hinton Agar
SHV	Sulphydril Variant
SIM	Simon Indole Motility
SOP	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
SSA	sub Saharan Africa
TEM	Temoniera (Name of Greek girl patient)
TSI	Triple Sugar Iron Agar
UTI	Urinary Tract Infection

ABSTRACT

Background: Extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* (ESBL-PE) are a group of bacteria that are resistant to a conventional therapy, in particular third generation cephalosporins, fluoroquinolones and penicillins. Currently, there are growing evidences indicating that drug resistant bacteria notably ESBL-PE is greatly implicated in immune-compromised groups namely individuals with HIV infection. However, little is known about the burden of ESBL-PE in places where HIV infection is rampant.

Objective: To assess the prevalence, antimicrobial resistance pattern of ESBL-PE among HIV/AIDS patients at the University of Gondar hospital, North-west Ethiopia.

Method: A cross-sectional study was conducted among HIV/AIDS patients seeking ART service at the University of Gondar Hospital ART clinic from February-May, 2017. Pre-tested and structured questionnaire was used to collect data on socio-demographic and clinical related factors. Clean catch mid-stream urine samples were collected and cultured in line with standard procedures. The drug susceptibility testing was performed by Kirby Bauer disc diffusion method. ESBL detection was performed using double disc synergy test and combined disc methods. Data entry and analysis was performed using SPSS version 20.

Result: Among a total of 387 HIV/AIDS patients, 42 (10.9%) *Enterobacteriaceae* uropathogens were recovered. Of these isolates, nine (21.4%) were ESBL producers. The highest prevalence of ESBL production was noted in *E.coli* (44.4%) followed by *K.pneumoniae* (22.2%) and *Enterobacter* spp. (22.2%). Higher drug resistance rates were observed among ESBL producing isolates compared to ESBL non producing isolates. The ESBL-PEs demonstrated no resistance to Nitrofurantoin, whereas high resistance rates were noted to Amox-clavulanic (100%), Ampicillin (95%), Cotrimoxazole (74%), cefotaxime (88.9%) and ceftazidime (88.9%). The overall prevalence of multidrug resistance of all isolates was 92.9% and all ESBL isolates were multidrug resistant

Conclusion and Recommendation: Considerably, high prevalence of ESBL-PE was observed in HIV patients. *E. coli* and *K. pneumonia* were the most prevalent ESBL producers. All ESB-PE isolates were MDR for all tested antimicrobial agents. Antibiotic resistance rates in ESBL-PE isolates were significantly higher than other ESBL non producing isolates Therefore, antimicrobial stewardship programs needed to be promoted for rational use of drugs especially in the management of HIV/AIDS patients.

Key words: Antimicrobial Susceptibility pattern, Extended Spectrum Beta lactamase (ESBL), Ethiopia, HIV.

1. INTRODUCTION

1.1. Background

Enterobacteriaceae are groups of non-sporulating, facultative anaerobic Gram negative bacteria, and are natural inhabitants of human and animal intestine, but some of them like *Salmonella* spps., *Shigella* spps. and *Yersinia* spp are human intestinal pathogens (1–3). Besides, they are usually found in soil, environment and on plants (4,5). These includes *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Morganella* spp., *Providentia* spp., *Enterobacter* spp., *Serratia* spp, s*almonella* spp., *Shigella* spp. and *Yersinia* spp. Especially, *Escherichia coli*, and *Klebsiella spps*. are the most implicated opportunistic pathogens, which frequently cause a variety of community and hospital acquired infections, including urinary tract infection (UTI), bacteremia, pneumonia, abdominal infections, meningitis, and wound infection, mainly in immuno-compromised population, notably in Human Immunodeficiency Virus (HIV) /AIDS patients (4).

Serious bacterial infections have emerged as an important cause of morbidity and mortality in individuals with HIV with case-fatality rates of up to 32% in patients with CD_4 count less than 200 cells/l (6,7). Peoples with HIV infection are more susceptible and increased rate of morbidity and mortality to bacterial infections because of defects in both cell-mediated and humeral immunity that leads for these patients to be exposed for multiple treatments. This multiple treatment finally facilitates for the emergence of drug resistant isolates via selective pressure as the survived strains are free to outnumber their population making the treatment ineffective. Moreover, many HIV patients are given primary cotrimoxazole prophylaxis that might increase the risk of antibiotic resistance in a variety of bacterial pathogens in high-risk population. As a result, management of bacterial infections in HIV patients is important for preventing further emergence of drug resistance (6–8).

Enterobacteriaceae are important human pathogens while increasing number of antibiotic resistant strains are detected worldwide. The most commonly observed resistances were against beta-lactams, fluoroquinolones, aminoglycosides and recently polymyxins (3). *Enterobacteriaceae* can develop several mechanisms to avoid the inhibitory effect of

antibiotics thus, becoming resistant. Nowadays, multi-drug resistant strains emerged, possessing several resistant mechanisms against different antibiotic groups (3).

Resistance mechanisms can be described in two ways: Intrinsic resistance mechanism whereby microorganisms naturally do not possess target sites or they naturally have low permeability for the drugs and acquired resistance mechanism whereby a naturally susceptible microorganism acquires resistance mechanisms through; production of hydrolyzing enzymes, mutation and post-transcriptional or post-translational modification in the targets, active efflux of the antimicrobial agent, overproduction of the targets and expression or suppression of a gene in vivo in contrast to the situation in vitro. Most importantly, resistance to beta-lactam antibiotics occurs by three mechanisms: failure of the beta-lactam to reach the penicillin-binding proteins (PBPs), low-affinity to the PBPs and inactivation of the drug by beta-lactamases. Among this, beta-Lactamases are the commonest cause of bacterial resistance to beta-lactam antimicrobial agents which hydrolyze the beta lactam rings (9,10).

Extended-spectrum beta-lactamases (ESBLs) are a plasmid mediated heterogeneous group of transferable beta lactamase enzymes that hydrolyze penicillins, first-, second-, and third-generation cephalosporins, aztreonam and other antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazoletrimetroprim but not cephamycins and carbapenems and also blocked *in vitro* by beta-lactamase inhibitors such as clavulanate, tazobactam and sulbactam (11–16). The first report of plasmid-encoded beta lactamases was published in 1983 in Germany. Later these enzymes were named extended-spectrum beta-lactamases (9,17).

Most ESBLs have evolved by point mutation of naïve TEM, SHV and recently CTX-M beta-lactamases with amino acid replacement, resulting for more than 150 variants of ESBL enzymes (9,18–20). The most common ESBLs types recognized currently are TEM (Temoniera)-beta-lactamases, SHV (sulphydril variable)-beta-lactamases, and CTX-M (Cefotaximase-Munich) beta-lactamases. Each of these enzymes derives from its own

progenitor. Interestingly, SHVs are more prevalent in Europe; TEMs are dominantly present in the USA while the CTX-Ms are being increasingly detected worldwide (3).

Extended spectrum beta lactamases producing *Enterobacteriaceae* (ESBL-PE) have spread widely and have become a major cause of nosocomial infections associated with high mortality rates, particularly in serious infections such as urinary tract infection (UTI). The widespread use of broad spectrum antibiotics which account for approximately 50% of global antibiotic consumption and, prolonged hospitalization, severe illnesses, co-morbidities like in case of HIV/AIDS, recent surgery, instrumentation, admission to an intensive care unit, combination of antibiotic therapy with a quinolone and empirical treatment without proper antibiotic susceptibility testing are the major causes for the development of multidrug resistant (MDR) infection with ESBL-PE (9,21–23).

Majority of ESBL-PE associated infections are resistant to various antibiotics, leaving only limited compounds as a choice of therapy. Presently, carbapenems became the first line antimicrobials for the treatment of such infections (24).

Proper infection control practices and barriers are essential to prevent spreading and outbreaks of ESBL producing bacteria. The reservoir for these bacteria seems to be the gastrointestinal tract of patients, oropharynx, colonized wounds and urine (25). Alternative reservoirs like contaminated hands and stethoscopes of healthcare providers are also important factors in spreading infection between patients (25). Essential infection control practices should include avoiding unnecessary use of invasive devices such as indwelling urinary catheters or IV lines, hand washing by hospital personnel, increased barrier precautions, and isolation of patients colonized or infected with ESBL producers (26).

1.2. Statement of the problem

Infectious diseases had been the leading cause of death until the middle of the 20th century or until the introduction of novel antibiotics in clinical medicine (27). The introduction of antibacterial agents was immediately accompanied by the emergence of drug resistant organisms which adapted different mechanisms to counter the effect of the antibiotics (28,29). In the fight against the drug resistant strains of bacterial pathogens, few potent drugs, including the third generation cephalosporins have been discovered and have been in use for the treatment of various bacterial infections (30). However, the continual emergence of drug resistant pathogenic bacteria, including the more potent third generation cephalosporin drugs made a great impediment to the rapid prognosis of patients that has been a major clinical problem in the treatment of various infections caused by members of the *Enterobacteriaceae* (31).

Infections caused by ESBL producing bacteria often involve immune-compromised patients (like in HIV patients), making it difficult to eradicate these organisms in high-risk wards, such as intensive care units (32,33). Extended spectrum beta lactamases strains have been associated with resistance to other non beta-lactam antibiotics like aminoglycosides and chloramphenicol. Another challenging property related to ESBL strains is that they might show a false sensitivity in vitro testing which leads to inappropriate antibiotic selection in infections caused by these organisms resulting for treatment failures, poor clinical outcomes, prolonged hospital stay, delayed initiation of appropriate antibacterial therapy, increased morbidity, mortality and health care costs (34–37).

In the past 2 decades, there has been an explosive increase in the prevalence of high-level resistance to beta-lactam antibiotics in members of Gram-negative *Enterobacteriaceae* (35). The current increase in ESBL producing bacteria in patients at the time of hospital admission points towards a continent-wide rise, mainly in *Escherichia coli*, with great variations in the occurrence and distribution of different ESBLs among countries and even among different regions of a country (38).

The global prevalence of ESBL producing organisms is presently increasing from <1% to 74% (39,40) that might be because of the global increasement of the prevalence of HIV/AIDS and other factors which contribute to immunosuppression. In the decade of the 1990s, ESBL producing isolates raised from 6% to 9% in United States, 10% to 35%, Latin America, 5.5% to 8%, Korea, 12 to 24, Thailand, 46% to 50% India (14,41–45) and a high prevalence of ESBL in Tanzania and South Africa which account 29% and 36.1% respectively (17,46). In Ethiopia, some local studies revealed that the magnitude of ESBL isolates are increasing from 38.4% to 52% (9,47).

This upward trend of ESBL-PE especially in sub-Saharan Africa (SSA) counties might be due to poor hygiene, unreliable water supplies, civil conflicts, and increasing numbers of immunocompromised people, such as those with HIV/AIDS, which facilitate both the evolution and their rapid spread in the community of resistant pathogens through selective pressure (48).

By the end of 2013, 66.7% of 6000 new global HIV infections each day, and 74% of 1.5 million AIDS related deaths from the world accounted in sub-Saharan Africa countries (49). This increased prevalence of HIV/AIDS infections, especially in sub-Saharan African countries, including Ethiopia has predisposed populations to the excessive increase in the utilization of antibiotics to prevent and treat opportunistic infections since these patients are highly exposed for a number of and frequent infections which raise concerns over the looming emergence and spread of resistance to cheap and well-tolerated antibiotics (48). For instance, in a survey on *E. coli* in Kenya, 27% were ESBL of which 57.8% were MDR (50), Guinea-Bissau, 32.6% ESBL producing *E. coli* and nearly all isolates were multidrug resistant (51) , Cameroon, 16% *E. coli* showed ESBL production (52), Gabon, 45% revealed ESBL carriage and most isolates were MDR strains (53). Due to limited capacity for disease detection and surveillance, the burden of illnesses due to treatable bacterial infections, their specific etiologies, and awareness of antibacterial resistance is less well established in most of SSA, and therefore, the ability to mitigate their consequences is significantly limited (48).

Studies have been conducted on the ESBL-PE strains in some part of Ethiopia like in Jimma (9), Harar (54), Addis Ababa (47) and Adama (31) on the prevalence of ESBL producing *E.coli* and *K. pneumoniae* that investigated for the existence and prevalence of ESBL producing isolates of *E.coli* and *K.pneumoniae*, but did not consider other *Enterobacteriaceae* species with ESBL producing isolates. Therefore, the present study was aimed to figure out the burden, antimicrobial resistance pattern of ESBL-PE isolates among HIV/AIDS patients.

1.3. Literature reviews

1.3.1. Epidemiology of ESBL Producing *Enterobacteriaceae*

The upward trend in the prevalence of pathogens producing ESBLs is of increasing clinical concern. Infections with these ESBL producing organisms continue to be associated with higher rates of mortality, morbidity and health care costs (55). The prevalence of ESBL producing bacterial pathogen varies from country to country and even locally within the same country (31,56).

Low prevalence of ESBL-PE has been reported in studies conducted in the USA (8.6%), and UK (1%). Both studies have also indicated that *E. coli* and *K. pneumoniae* were the first and the second ESBL producing bacteria (57,58). Relatively, the high burden of ESBL-PE has been implicated in Asian countries, ranges from 22.9% to 69% (55,59–61). The most common ESBLPEs were *K. pneumoniae* (36 to 71.4%), *E. coli* (10.8% to 60%), and *K. oxytoca* (4 to 28.6%). *E. coli* that produce ESBL showed maximum susceptibility to imipenem (100%), followed by piperacillin-tazobactum (84%), amikacin (68%), gentamicin (9%), ciprofloxacin (9%) and amoxicillin-clavulanic acid (7%). Similarly, ESBL producing *K. pneumoniae* showed a very good susceptibility to imipenem (98%), followed by piperacillin-tazobactum (68%), amikacin (40%), gentamicin (15%), ciprofloxacin (15%) and amoxicillin-clavulanic acid (5%).

Several studies have been undertaken in different settings of African region. A finding from Nigeria among HIV/AIDS patients revealed that 17.3% were positive for ESBL production, 28.9% were from *E. coli,* 20.0% from *K. pneumoniae,* 15.6% from *P. aeruginosa,* 11.1% from S*erratia marcescens,* 6.7% from *Salmonella* spp., 11.1% from *Proteus* spp., 4.4% from *Citrobacter* spp. and 2.2% from *Enterobacter* spp. (62). Comparatively, high prevalence of ESBL-PE (49.3%) has been reported in Ghana. The prevalence within each species was the highest among *Enterobacter cloacae*, 75.0%, followed by *K. pneumoniae*, 61.5%, *C. freundii*, 50%, *K. oxytoca*, 45.0% and *E. coli*, 43% (63). Similarly a high prevalence of ESBL-PE (62 %) was reported in Uganda, Mulago Hospital. *Escherichia coli* was the most

isolated organism (53.9 %), followed by *K. pneumoniae* (28.7 %). A higher percentage of isolates were showing resistance to ceftazidime (73 %) compared to cefotaxime (57.5%) (64). Similar study in 3 main Burkinafaso hospitals has revealed that 58 % were identified as potential ESBLs-PE of which 67.5 % *E. coli*, 26 % *K. pneumoniae*, 4 % *Enterobacter cloacae*, 1 % *Providencia stuartii*, 0.5 % *Enterobacter aerogenes*, 0.5 % *Citrobacter freundi* and 0.5 % *Morganella morgannii* species (65).

There are few studies conducted in Ethiopia on ESBL-PE. A finding from Adama Hospital, Ethiopia reported that 25% were ESBL producers from *Enterobacteriaceae* isolates of which *E. coli* was the leading ESBL producer (28.57%) while *Proteus* species, *Klebsiella* species, *E. cloacae* and *Citrobacter* species accounted for 33.3%, 25%, 33.3% and 33.3% respectively (31). Similar study in Jimma University Specialized Hospital, South-West, Ethiopia has showed that 38.4% ESBL-PE of which 28.2% was *E. coli* and 70.4 % was *K. pneumoniae* (9).

A high prevalence (52%) of gastrointestinal carriage rate of ESBL-PE was investigated in Tikur Anbesa Specialized Hospital of which, *E. coli* and *K. pneumoniae* accounted 68% and 32%, respectively (47). It was also found that 33.3% of *Klebsiella* isolates were ESBL-PE from *Klebsiella* spp. isolates in clinical samples in a study conducted in four different hospitals of Harar region. *Klebsiella* spp. resistance pattern was found against cephalosporins: cefotaxime (39%), cefoxitin (39%), ceftazidime (40%), ceftriaxone (40%), cephalothin (42%), chloramphenicol (70%), gentamicin (61%) and trimethoprim-sulphamethoxazole (65%). Multi-drug resistant isolates were more prevalent among the ESBLs producers (95%) than non-producers (53%) (54).

Regarding with MDR patterns of *Enterobacteriaceae* isolates, different studies in the world has been depicting that the rate of multidrug resistance (MDR) is increasing. A study from different areas like USA and Nepal has shown the prevalence of 19.1% and 41.1% respectively (66,67). Additionally, a high prevalence of MDR was reported from Mozambique (88.2%) among *Enterobacteriaceae* (68).

Moreover, very high prevalence of MDR was reported in different areas of Ethiopia in different times with a prevalence of 90.8% from Hawasa (69), 100% from Jimma (70), 75.2% from Dessie (71), 92.2% from Bahir Dar (72); 87.4% and 93.5% from Gondar (73,74) .

1.3.2. Risk factors for ESBL producing *Enterobacteriaceae* infection

Various studies have been conducted on epidemiology and risk factors associated with infections with ESBL producing bacteria. The most recognized risk factors for ESBL infections includes age, gender, prolonged hospital stay, surgical intervention, irrational antibiotic use, central venous catheterization, residence in a long-term care facility, chronic disease such as diabetics, HIV, and liver disease (75,76). The link between previous hospitalization and ESBL infection was previously reported. For example, the prevalence of ESBL infection among hospital admitted patients was reported 45.9% in Turkey among children (77). More than that, a higher prevalence (50.5%) of ESBL infection was reported in Israel (78).

Recent surgery, antibiotic use within the 30 days preceding the infection and presence of urinary catheter were significantly associated with an increased risk for infection with an ESBL-producing organism from a study conducted in Lebanon (79). Additionally, recent treatment in a foreign country, previous antibiotic therapy and Mechanical ventilation increased the risk of infections due to ESBL producing *E. coli* or *K. pneumoniae* in a study conducted in Switzerland (80). In a study from Barcelona also found that acquisition of resistant or potentially resistant microorganisms had a significantly lower CD4 cell count than those who did not acquire this microorganism in HIV-infected patient (81).

Transfer of patients from another ICU, hospital admission in another country, surgery within the past year, prior neurologic disease, and prior administration of third generation cephalosporin (within 3–12 months before ICU admission) were found to be independent predictive factors of colonization by ESBL from a study in France hospital ICU patients (82). Similarly, a study in Israel upon hospital admitted patients revealed that nursing home

residency, prior hospitalization, prior antibiotic treatment and prior ESBL carriage were risk factors for ESBL infection (78).

Previous use of antibiotics, urinary catheter, mechanical ventilation, previous hospitalization and nosocomial origin of infection were significant risk factors for acquiring ESBL infection from a study conducted in Saudi Arabia (83). A multinational survey conducted in Europe, Asia, and North America revealed that recent antibiotic use, residence in a long term care facility, recent hospitalization, age >65 years, and male sex were the significantly associated risk factors (84).

A study in Ghana showed that distribution of ESBL producing isolates was compared across age groups, ESBL prevalence was significantly higher among isolates from patients at extremes of ages, specifically neonates (65.1%) and adult patients > 65 years of age (70.5%) (63). In Ethiopia, treatment with third generation cephalosporin was identified as a sole risk factor for acquisition of ESBL enzyme from a study in Jimma University specialized Hospital (9).

1.4. Significance of the Study

The increased predisposition of HIV/AIDS patients to invasive bacterial isolates has been described, but there are no detailed and published data or literatures on the prevalence of ESBL-PE in HIV/AIDS infected individuals in Ethiopia. Overuse of antimicrobial agents is strongly related to HIV/AIDS infection leading to rapid occurrence of multi-drug resistance to the third generation cephalosporins, aminoglycosides and fluoroquinolones that is indicative of the emergence of ESBL producing Gram negative bacilli. Therefore, this study was designed to look at the existence and magnitude of ESBL-PE among HIV/AIDS patients.

In line with this study, the findings will provide baseline information for health sector administrators and concerned bodies in planning and managing of drug resistance. Specifically, to promote specific treatment and preventive measures such as rational antibiotic use policy and plan a proper hospital infection control strategy in prevention of the dissemination of these strains. It also gives information for physicians for empirical treatment

in areas where there is no routine ESBL detection. Furthermore, limiting drugs to those that act against the right organisms will help to conserve their efficaciousness which further reduces the rate of morbidity, disability and mortality.

2. OBJECTIVES

2.1. General objective

- ✓ To assess the prevalence, antimicrobial susceptibility pattern of ESBL producing *Enterobacteriaceae* among HIV/AIDS patients

2.2. Specific objectives

- ✓ To determine the prevalence of ESBL producing *Enterobacteriaceae* among patients with HIV/AIDS
- ✓ To determine drug susceptibility patterns of ESBL producing *Enterobacteriaceae* isolated from HIV/AIDS patients
- ✓ To determine multidrug resistance patterns of *Enterobacteriaceae* isolated from HIV/AIDS patients

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted at the University of Gondar Hospital, North Gondar zone of Amhara Regional State, North west Ethiopia. University of Gondar Hospital is found in Gondar town at a distance of 747 km from the capital city of the country, Addis Ababa and 182 km from Bahir Dar, the capital city of Amhara regional state. The hospital provides services for at least 5 million populations living in and around Gondar including ART services for HIV/ AIDS patients. Currently, ART clinic provides services for 5094 ART patients and 141 pre-ART patients.

3.2. Study design and period

A Hospital based cross-sectional study was conducted among HIV/AIDS patients from Feb-May, 2017.

3.3. Population

3.3.1. Source population

The source population was all HIV/AIDS patients seeking health services at the University of Gondar Hospital.

3.3.2. Study population

The study population was all HIV/AIDS patients seeking ART services during the study period at the University of Gondar Hospital ART clinic and willing to give consent.

3.4. Inclusion and exclusion criteria

3.4.1. Inclusion criteria

Patients who have regular ART follow up were included in the study

3.4.2. Exclusion criteria

Patients with a history of antibiotic use for the past seven days, other than ART were excluded.

3.5. Study Variables

3.5.1. Dependent variable

- Presence ESBL producing *Enterobacteriaceae*
- Drug susceptibility pattern

3.5.2. Independent variables

Age, sex, educational status, occupation, residence, previous hospitalization, previous surgical procedure, previous exposure to antibiotics, cotrimoxazole prophylaxis therapy and adherence, ART status, antibiotic use without physician prescription (self prescription), use of oral contraceptives, level of CD_4 count, and AIDS stage.

3.6. Sample size determination and sampling technique

3.6.1. Sample size determination

The sample size was determined using a single population proportion formula as follows:

$N= z^2 p (1-p)/ d^2$; where: N = The number of study subjects from which samples will be taken; Z = Standard normal distribution value at 95% CI, which is 1.96; P = the prevalence of ESBL-PE infection taken as 50% (0.5) since there is no previous study report in Ethiopia exactly on HIV/AIDS patients. d = the margin of error taken as 5%.

Accordingly, the sample size was:

$$\mathbf{n_i} = (1.96^2 \text{x} 0.5 \text{x} 0.5) / 0.0025 = \underline{384}$$

By considering a 10% non-response rate, $\mathbf{n_f} = \mathbf{n_i}$+10% of $\mathbf{n_i} = \underline{423}$

3.6.2. Sampling technique

On average 40 ART patients/day, 880 patients/month and 2640 individuals were served for three months within the study period, which was taken as a sampling frame (N). So, K value was N/nf= 6. Then, the study subjects were selected from 1st six subjects randomly and other subjects were incorporated in the study for every six of patients by their appearance to the ART clinic using a systematic random sampling technique. For study participants who were not willing and illegible to participate, the collection procedure was continued with that interval for every six cases using the non-response rate till the desired amount of sample was collected. Some important data related to patient information like CD_4 count of the study participants were obtained from the participant chart.

3.7. Operational definitions

Previous antibiotic use: Receipt of any systemic antibiotic for >48 hrs for the last 12 months before 7 days from data collection.

Level of CD4 count: - CD_4 count is the measure of the number of T cells expressing CD4 which indicate the immune function status in patients who have HIV infection that used for deciding when to begin antiretroviral treatment for newly screened patients during HIV infection and in this study the cutoff value was considered<500 cells/mm3 based on the current national guideline.

Previous hospitalization: - In this study previous hospitalization of the patient was considered if the patient was admitted in the hospital within one year before data collection.

Previous surgery history:-In this study previous history of surgery of the patient was considered if the patient had a history of any invasive surgery within 6 months before data collection.

Multidrug resistance: - Is resistance to one or more agents in three or more different classes of antimicrobials that the isolate is expected to be susceptible (76)

3.8. Data collection and Laboratory methods

3.8.1. Questionnaire

Data on socio-demographic characteristics and ESBL-PE related factors from each study participant was collected by a trained data collectors using pretested questionnaire guided interview.

3.8.2. Sample collection and Transport

Urine sample was collected from a total of 387 HIV patients at the University of Gondar Hospital ART clinic. Midstream Clean Catch urine samples were collected from study subjects using a sterile wide mouthed container after the participants were given appropriate instruction on how to collect urine samples and then sent to the laboratory immediately after collection using a cold chain.

3.8.3. Isolation and identification

The standard microbiological techniques was used for isolation and identification of bacterial isolates (61). For isolation and identification, the following media were used: MAC, CLED, TSI, LIA, SIM, urea, citrate and lysine decarboxylase. Urine specimen was inoculated on CLED agar media using a calibrated wire loop (0.001ml). These inoculated plates were incubated in an aerobic atmosphere at 35-37^{O}C for 18-24 hrs. All significantly positive cultures were then characterized by a colony characteristic appearance and tested by Gram stain. Members of the family *Enterobacteriaceae* of concern were identified by a series of

biochemical tests by testing microorganism's indole production, lactose fermentation, H_2S production, citrate utilization, motility test, urease test and lysine decarboxylase test.

3.8.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on each bacterial isolates using Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). Briefly, 3–5 pure colonies of each bacterium was picked and transferred to a tube containing 5 ml sterile nutrient broth (Oxoid) and then mixed thoroughly to make the suspension homogeneous and the suspension was adjusted to 0.5 McFarland turbidity standard (Bacterial concentration of 1.5×10^8 colony forming unit/ml) by further addition of colony or incubating suspension at 37°C until the turbidity of the suspension adjusted to a 0.5 McFarland turbidity standard for less turbid suspensions or by adding a sterile nutrient broth aseptically for over turbid suspensions (85).

A sterile swab was dipped in the suspension and the entire surface of the Muller Hinton agar plate was uniformly flooded with the suspensions and allowed to dry for about 3-15 minutes. The antimicrobial impregnated discs were placed in the media using sterile forceps in such a way that each disc were placed at least 24 mm away from each other to avoid the overlapping of zone of inhibition. After the discs were placed on the inoculated media, the plate was allowed to stand for 15 minutes, so that the antibiotic diffused into the media. The plates then incubated at 37^OC for 18- 24 hrs and observed for the zone of inhibition (86). Grades of the susceptibility pattern was recognized as sensitive, intermediate and resistant by comparison of zone of inhibition with current standards of clinical laboratory standards institute (CLSI) guideline (85). The following antibiotics discs were used in the sensitivity test; cefuroxime (30 μg), cefexime (30 μg), cefotaxime (30 μg), cefpodoxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), nitrofurantoin (100 μg), cotrimoxazole (25 μg), amox-clavulanate (20/10 μg), Amikacin (30 μg) and ampicillin (10μg).

3.8.5. Testing of the ESBL production

3.8.5.1. Screening test for ESBL detection

Screening test for ESBL detection was done according to the CLSI guidelines. Isolates showing inhibition zone size below the CLSI stated break points was considered a potential ESBL-producer: Cefpodoxime $\leq$ 22 mm, Ceftazidime $\leq$ 22 mm, Cefotaxime $\leq$ 27 mm and Ceftriaxone $\leq$ 25 mm. The sensitivity of screening test for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection as the former drug screens for the presence of CTX-M, SHV and TEM derivatives and the later screens for SHV and TEM derivatives (87,88).

3.8.5.2. Phenotypic Confirmatory test

Extended spectrum beta lactamase detection was carried out by double disc synergy tests (DDST) using third generation cephalosporins and modified double disc synergy test (MDDST) using cefepime along with the third generation cephalosporins. A negative DDST or MDDST was confirmed with combined disc test method.

A. Double disc synergy test (DDST)

The double disc synergy test was used as primary isolation method to identify the ESBL producing organisms. A 0.5 McFarland's standard suspension of the test isolates was inoculated by using a sterile cotton swab on the surface of a Muller Hinton Agar plates. Antibiotic discs of amoxicillin-clavulanic acid (20/10µg) at the center; cefotaxime (30µg) and ceftazidime was placed at a distance of 15 mm apart and incubated center to center. After incubating overnight at 37˚C that showed a clear extension of cefotaxime and ceftazidime inhibition zones towards the disc containing clavulanic acid was considered ESBL producer. Isolates which were screened and found positive for ESBL production may be negative for confirmatory test using DDST due to the coproduction of other beta lactamases like AMPC

lactamase. In such cases, modified double disc synergy test was used using Cefepime antibiotics for inhibiting the effect of other beta lactamases (AMPC lactamase).

B. Modified double disc synergy test (MDDST)

Modified double disc synergy test was employed in case the double disc synergy test yields a negative result as if the screening test has been positive. The test was performed by using a disc of amoxicillin-clavulanate (20/10 μg) along with four cephalosporins (cefotaxime, ceftriaxone, ceftazidime and cefepime). A lawn culture of the organisms was made on a Mueller-Hinton agar plate. A disc which contained amoxicillin-clavulanate (20/10 μg) placed in the center of the plate. The discs of third generation cephalosporin and fourth generation cephalosporin was placed 15 mm and 20 mm apart respectively, center to center to that of the Amoxicillin clavulanate disc. Any distortion or increase in the zone towards the disc of amoxicillin-clavulanate was considered positive for the ESBLs production. For a negative MDDST as a result of the effect of coproduction of other enzymes, combined disk test was employed.

C. Combined disc test

Discs containing 30 μg of cefotaxime, ceftazidime, or cefepime, and discs containing a combination of the three drugs plus 10 μg of clavulanic acid were placed independently, 30 mm apart, on a lawn culture of 0.5 McFarland standard of the test isolate on a Mueller-Hinton agar plate and incubated for 18-24 hours at 35°C. Depending on the disc type, a difference of ≥5 mm between inhibition zone size of the cephalosporin with clavulanate disc and cephalosporin alone or a zone expansion of 50% in cephalosporin with clavulanate disc were considered indicating ESBL production (89–91).

3.9. Quality control

The reliability of the study findings was guaranteed by implementing Quality Control (QC) measures throughout the whole process of the laboratory work. All materials, equipments and procedures were adequately controlled. Pre-analytical, analytical and post-analytical stages of quality assurance and standard operating procedures (SOPs) were strictly followed. Pre-tested questionnaire guided interview was used for data collection on socio-demographic characteristics and associated factors. Sterility of culture media was checked by incubating 5% of the batch at 35-37^{0}C overnight and was evaluated for possible contamination. The standard reference bacteria strains of *K. pneumoniae* (ATCC®700603) was used as positive control and *E. coli* (ATCC®-25922) was used as negative control of ESBL detection test (36,87). Moreover, the whole procedure and result interpretation was cross checked by senior laboratory professionals and the potency of antibiotics were monitored using control strains based on the following criteria:

Table 1**:** Criteria for determining the potency of the test antibiotics

ESBLs control strain	E. coli ATCC 25922	K.pneumoniae ATCC 700603
Ceftazidime 30 μg	25-32 mm	22-29 mm
Cefotaxime 30 μg	29-35 mm	18-22 mm

3.10. Data organization, processing and analysis

Data was collected, summarized, tabulated and analyzed using SPSS version 20 software. The statistical significance association was measured by using the Chi-square test and P value < 0.05 was considered statistically significant.

3.11. Ethical consideration

Ethical clearance was obtained from University of Gondar, School of Biomedical and Laboratory sciences, ethical review committee and official letter of co-operations was provided to the University of Gondar Hospital prior to data collection. Written informed consent was obtained from study participants, guardians or caretakers of children after explaining the purpose and objective of the study. Any patient, who was unwilling to participate in the study, was not forced to participate. They were informed that all data and sample obtained from them was kept confidential by using codes instead of any personal identifiers and is meant only for the purpose of the study. Laboratory results from the study participant were communicated to their physicians for appropriate treatment or management.

4. RESULTS

Socio-demographic characteristics

A total of 387 HIV/AIDS patients were enrolled in the study. The majority of study participants were females 273 (71.1 %) with male to female ratio of 1:2.3. The mean (SD) age of the study participants was 37.9 ± 9.7 years. Most of the respondents were in the age group of 30-40 years, 159 (41.1%). Besides, 358 (92.5%) of the participants were urban residents and 210 (54.3 %) were also participants who had educational level of primary school and below (Table 2)

Table 2: Socio-demographic characteristics of HIV/AIDS patients at the University of Gondar Hospital, Feb-May, 2017

Characteristics		Frequency	Percent
Sex	Male	112	28.9
	Female	275	71.1
Age	<30	102	26.4
	30-40	159	41.1
	>40	126	32.6
Ethnicity	Amhara	380	98.7
	Tigray	5	1.3
Religion	Orthodox	355	91.7
	Muslim	26	6.7
	Protestant	6	1.6
Residence	Urban	358	92.5
	Rural	29	7.5
Occupation	Unemployed	12	3.1
	Daily laborer	21	5.4
	Housewife	144	37.2
	Merchant	93	24.0
	Farmer	20	5.2
	Employed	97	25.1
Educational status	Primary school and below	210	54.3
	Secondary school	108	27.9
	College/University	69	17.8

Distribution of *Enterobacteriaceae* in patient characteristics

A total of 387 urine samples were collected from HIV/ADS patients and analyzed accordingly. Of these, 42 (10.9%, 95% CI; 7.8-14.3) patients had positive culture for *Enterobacteriaceae* result with a single non-duplicate isolates. In brief, culture positivity rate in patients with a history of prior antibiotic use was significantly higher than from those who were not (57.1% versus 42.9%, P<0.001). Likewise, a significant high bacterial isolation rate was observed in patients with history of antibiotic use without prescription and surgery for the past six months (Table 3). As presented in table 4, the most common isolated organisms were *E.coli* 29 (69%), followed by *Enterobacter* spp six (14.3%) and *K.pneumoniae* four (9.5%). Most importantly, among the isolated *Enterobacteriaceae*, nine (21.4 %, 95% CI: 11.9-31) isolates were found to be ESBL producers with highest prevalence of *E.coli*, four (44.4%) followed by *K.pneumoniae*, two (22.2%), *Enterobacter* spp. two (22.2%).

Table 3: Distribution of *Enterobacteriaceae* per patient characteristics at the University of Gondar Hospital, Feb-May, 2017

Variables		Culture Positivity (*Enterobacteriaceae*)		
		Negative N (%)	Positive N (%)	P-value
Sex	Male	96 (85.7)	16 (14.3)	0.166
	Female	249 (90.5)	26 (9.5)	
Age	<30	94 (92.2)	8 (7.8)	0.158
	30-40	136 (85.5)	23 (14.5)	
	>40	115 (91.3)	11 (8.7)	
Occupation	Unemployed	12 (100)	0	0.805
	Daily laborer	19 (90.5)	2 (9.5)	
	Housewife	126 (87.5)	18 (12.5)	
	Merchant	82 (88.2)	11 (11.8)	
	Farmer	17 (85)	3 (15)	
	Employed	89 (91.8)	8 (8.2)	
Educational status	Primary and below	187 (89)	23 (11)	0.480
	Secondary school	95 (88)	13 (12)	
	College/University	63 (91.3)	6 (8.7)	
Previous hospitalization for the past 12 months	No	328 (91.9)	29 (8.1)	
	Yes	17 (56.7)	13 (43.3)	<0.001
Previous UTI history	NO	283 (90.4)	30 (9.6)	0.099
	Yes	62 (83.8)	12 (16.2)	
Previous antibiotic history	No	287 (94.1)	18 (5.9)	<0.001
	Yes	58 (70.7)	24 (29.3)	
Antibiotic use without prescription	No	330 (90.2)	36 (9.8)	0.007
	Yes	15 (71.4)	6 (28.6)	
Surgery history in the last 6 months	No	329 (90.1)	36 (9.9)	0.011
	Yes	16 (72.7)	6 (27.3)	
Pregnancy	No	239 (90.9)	24 (9.1)	0.519
	Yes	12 (85.7)	2 (14.3)	
Use of contraceptive	No	208 (91.6)	19 (8.4)	
	Yes	43 (86)	7 (14)	0.217
Cotrimoxazole prophylaxis	No	47 (85.5)	8 (14.5)	0.342
	Yes	298 (89.8)	34 (10.2)	
Adherence of cotrimoxazole	Not completed	68 (93.2)	5 (6.8)	0.279
	Completed	230 (88.8)	29 (11.2)	
ART status	Pre-ART	16 (80)	4 (20)	0.177
	On ART	329 (89.6)	38 (10.4)	
WHO AIDS staging	Stage I	124 (90.5)	13 (9.5)	0.842
	Stage II	218 (88.3)	29 (11.7)	.
	Stage III	2 (100)	0	
	Stage IV	1 (100)	0	
CD4 count	<=499	191 (88.4)	25 (11.6)	0.608
	>=500	154 (90.1)	17 (9.9)	

Table 4: Percentage of ESBL-PE and non ESBL-PE isolates from HIV/AIDS patients at the University of Gondar Hospital, Feb-May, 2017

ESBL enzyme production	Bacterial isolates					
	E.coli N (%)	*K.pneumoniae* N (%)	*Enterobacter Spp.* N (%)	*K.ozanae* N (%)	*K.oxytoca* N (%)	Total N (%)
Producer	4 (44.4)	2 (22.2)	2 (22.2)	1 (11.2)	0	9 (100)
Non producer	25 (75.8)	2 (6.1)	4 (12.1)	1 (3)	1 (3)	33 (100)
Total	29 (69)	4 (9.5)	6 (14.3)	2 (4.8)	1 (2.4)	42 (100)

Antimicrobial Resistance pattern of isolated organisms

The overall antimicrobial resistance pattern of isolates has been presented in Table 5. Of the tested antibiotics, all isolates were found to be resistant to Amox-clavulanic acid; nearly 95% and 74% of the isolates were also resistant to Ampicillin and Cotrimoxazole, respectively. However, the isolates were 100% susceptible to Nitrofurantoin and comparatively, low resistance rate was observed to Ceftriaxone (2.4%) and Ciprofloxacillin (2.4%). Besides, resistance rate at species level has been also noted in Table 5. More than 62% *E.coli* isolates were resistant to cefotaxime, cotrimoxazole, ampicillin and amox-clavulanic acid. Relatively, low resistance rate was revealed in cefepime (3.4%), ciprofloxacillin (3.4%) and cefuroxime (6.9%). All *E.coli* isolates were sensitive for nitrofurantoin, amikacin, cefpodoxime and ceftriaxone. More than 50 % of *Enterobacter* spps exhibited resistance to cefotaxime, ceftazidime, cotrimoxazole, ampicillin and amox-clavulanic acid. Similarly, more than 75% of *K.pneumoniae* isolates posses a resistance character to cefotaxime, ceftazidime, cotrimoxazole, ampicillin and amox-clavulanic acid

Table 5: Antibiotic resistance pattern of isolated organisms from HIV/AIDS patients at the University of Gondar Hospital, Feb-May 2017

Drugs	**Bacterial isolates**					
	E.coli	*Enterobacter spp*	*K.pneumoniae*	*K.ozanae*	*K.oxytoca*	Total
CRX	2 (6.9)	0	1 (25)	0	0	3 (7.1)
CXM	3 (10.3)	1 (16.7)	1 (25)	0	0	4 (9.5)
CTX	18 (62.1)	3 (50)	3 (75)	2 (100)	0	26 (61.9)
CAZ	9 (31)	3 (50)	3 (75)	0	0	15 (35.7)
CTR	0	0	0	1 (50)	0	1 (2.4)
CPD	0	1 (16.7)	1 (25)	0	0	2 (4.8)
CFP	1 (3.4)	1 (16.7)	1 (25)	0	0	3 (7.1)
CPR	1(3.4)	0	0	0	0	1 (2.4)
COT	19 (65.5)	5 (83.3)	4 (100)	2 (100)	1 (100)	31 (73.8)
AMP	27 (93.1)	6 (100)	4 (100)	2 (100)	1 (100)	40 (95.2)
AMC	29 (100)	6 (100)	4 (100)	2 (100)	1 (100)	42 (100)
NIT	0	0	0	0	0	0
AMK	0	0	1(25)	1(50)	1(100)	3(7.1)

Note: Data are in number (%) unless and otherwise indicated CRX: Cefuroxime, CXM: Cefexime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, CPD: Cefpodoxime, CFP: Cefepime, CPR: Ciprofloxacillin, COT: Cotrimoxazole, AMP: Ampicillin, AMC: Amox-clavulanic acid, NIT: Nitrofurantoin, AMK: Amikacin

Multidrug resistance pattern

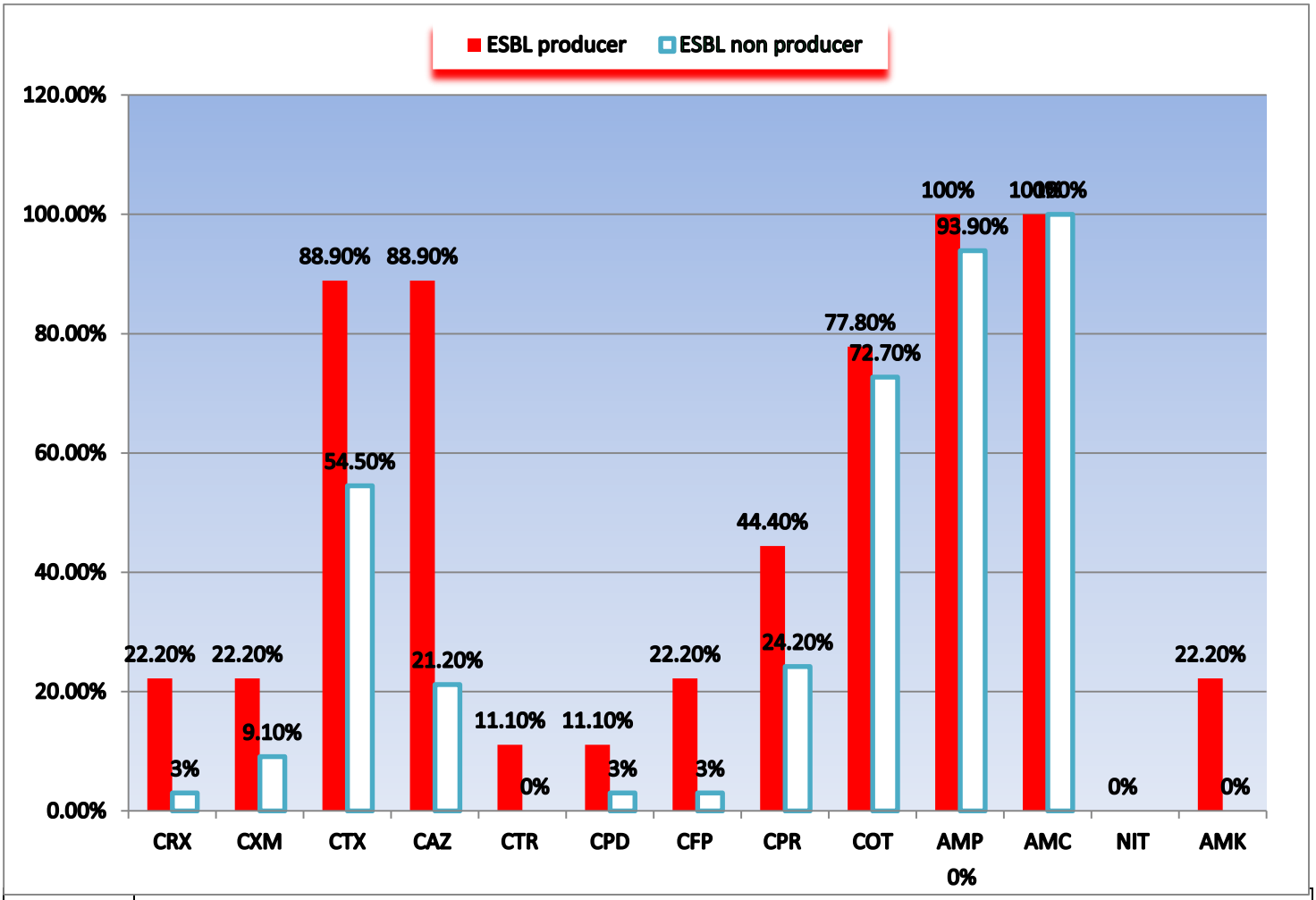
Interestingly, 39 (92.9%; 95% CI: 88.1-97.6%) of isolates showed resistance to three and more classes of antibiotics and which were multidrug resistant (MDR) organisms. Only three (7.1%) of isolates were found to be non-MDR strains (resistance either for only one or two classes of drugs). Briefly, six (100%) of *Enterobacter* spp., four (100%) of *K.pneumoniae,* two (100%) *K.ozanae,* one (100%) of *K.oxytoca* and 26 (89.7%) of *E.coli* isolates were MDR isolates among all isolates (Table 6).

Table 6: Multidrug resistance pattern of Enterobacteriaceae among HIV/AIDS patients at the University of Gondar Hospital, Feb-May 2017

Bacterial isolates	**Degree of antibiotic resistance of isolates**					
	R2	**R3**	**R4**	**R5**	**R6**	**MDR (≥ R_3)**
E.coli (N=29)	3 (10.3)	5 (17.2)	10 (34.5)	8 (27.6)	3 (10.3)	26 (89.7)
K.pneumoniae (N=4)	0	0	1 (25)	2 (50)	1 (25)	4 (100)
Enterobacter Spp. (N=6)	0	1 (16.7)	3 (50)	0	2 (33.3)	6 (100)
K.ozanae (N=2)	0	0	1 (50)	0	1 (50)	2 (100)
K.oxytoca (N=1)	0	1 (100)	0	0	0	1 (100)
Total (N=42)	3 (7.14)	7 (16.67)	15 (35.71)	10 (23.81)	7 (16.67)	39 (92.86)

Note: MDR: multidrug resistance (R≥ 3 classes**)**; R2: Refers to the resistance of isolates for one and two drugs; R3, R4 and R5: Refers to the resistance of isolates for 3, 4 and five drugs; R6: Refers to the resistance of isolates for 6 and above drugs

Moreover, the total resistance pattern of ESBL-PE isolates are presented in the fig.3. All ESBL producing isolates were 100 % resistant to ampicillin and amox-clavulanic acid and 88.9 % resistance to cefotaxime and ceftazidime, but least resistance was observed for both ceftriaxone and cefpodoxime. All ESBL-PE isolates were MDR for tested antimicrobials and 30 (90.9%) of non ESBL-PE isolates were MDR. Antibiotic resistance rate of ESBL-PE isolates was significantly higher than non-ESBL-PE strains for some of tested antimicrobials like cefuroxime (22.2% versus 3%; p=0.048), ceftazidime (88.9% versus 21.2%; p<0.001), cefepime (22.2% versus 3%; p=0.048) and Amikacin (22.2% versus 0%; p=0.006). But, significant variation was not observed for the following antibiotics; cefexime, cefotaxime, ceftriaxone, cefpodoxime, ciprofloxacillin, cotrimoxazole, ampicillin, amox-clavulanic acid and nitrofurantoin



	Antibiotics												
	CRX	CXM	CTX	CAZ	CTR	CPD	CFP	CPR	COT	AMP	AMC	NIT	AMK
p value [a]	**0.048**	0.281	0.060	**<0.001**	0.053	0.313	**0.048**	0.453	0.760	0.449	-	-	**0.006**

Figure 1: Antibiotic resistance rate of ESBL-PE isolates in comparison with non ESBL-PE isolates among study participants at the University of Gondar Hospital, Feb-May, 2017

5. DISCUSSION

It is a known that HIV/AIDS infection complicates the treatment of infection by increasing morbidity, mortality and increasing the risk of recurrent infections after treatment has been successfully completed. It is largely understood that HIV infection down regulates for cell mediated immune response during bacterial infection. The viral infection also predisposes loss of integrity of natural mucosal barriers and subsequently leads to high rate of bacterial infection. Therefore, the HIV/AIDS patients are highly vulnerable for repeated opportunistic infection which intern exposes for repetitive antimicrobial treatments as well as increased rate of hospitalization. As a result, these patients stand to the front line to be exposed for multidrug resistant organisms or to develop these organisms through selective pressure due to repeated exposure for antibiotics (62).

The upward trend in the prevalence of pathogens producing ESBLs is of increasing clinical concern related with the increased prescription of beta lactam drugs. Infections with these drug resistant strains continue to be associated with higher rates of mortality, morbidity and health care costs (55). Global reports have shown that considerable differences exist in the occurrence and proportion of ESBL-producing isolates in different countries. Besides, the overall epidemiology of drug resistant strains namely ESBL-PE doesn't well appreciated in resource limited countries (31,56).

Among the study participants, the overall prevalence of ESBL-PE infection was found to be 21.4% which is comparable with reports indicated in Jordan (22.9%) (61) Saudi Arabia (22%) (83) and Nigeria (17.3) (62). However, the prevalence noted in the present study was significantly higher compared with that documented in studies from USA (8.6%) (57), UK (1%) (58). On the other hand, it was lower than reports from different Asian countries like United Arab Emirates (41%) (55), India (38.83%) (60) and African countries like Ghana (49.3%) (63), Uganda (62 %) (64), Burkinafaso (58%) (65). The variation might be due to the fact that difference in sampling population, policy of antibiotics prescription, socio-cultural and economic factors.

In Ethiopia, there are still limited evidences regarding with the prevalence ESBL-PE. A report claims that on the prevalence of ESBL-PE in Adama, Ethiopia was 25% (31), which is comparable with the finding of the current study. However, in studies from other part of Ethiopia like, Jimma (38.4) (9), Addis Ababa (52%) (47) and Harar (33.3%) (54) had reported a higher prevalence of ESBL-PE than the current study. Lower prevalence of ESBL-PE of the current study compared with aforementioned reports might be related with variability of sampling population and methodological difference. Additionally, the present study reports the prevalence among HIV patients with both symptomatic and asymptomatic UTI cases that could underestimate the result.

Although there are differences in proportion, several studies had reported that *E. coli* and *K. pneumoniae* (9,47,55,58,62,64,83) were the most prevalent pathogens among the ESBL producing isolates in different geographical regions in agreement with our study where *E.coli* (44.4%) followed by *K.pneumoniae* (22.2%) and *Enterobacter spp* (22.2%) were predominantly isolated pathogens. The varying proportion of isolates with regarding to production of ESBL in different studies in different areas might be attributed with variation in geographical distribution of isolates, sample size, detection methodology used in each investigation and target population. The highest prevalence of *E.coli* isolation among total isolates in HIV/AIDS in UTI cases might be related with its virulence factor that *E.coli* has a virulence factor that will enable to attach to the walls of the renal tubules through Pilli so that will not be removed with renal flow rather moves to the opposite direction of the flow of urine. As a result, *E.coli* will have a high proportion of isolation in UTI cases.

In the current study, very high resistance of microorganisms was reported to Amox-clavulanic acid (100%), followed by Ampicillin (95.2%), Cotrimoxazole (73.8%), Cefotaxime (61.9%) and Ceftazidime (35.7%) among all isolates in agreement with other report (9). This high resistance rate to these drugs might be related with a high rate of prescription of these drugs as a routine therapy and as a prophylaxis therapy in case of cotrimoxazole in HIV/AIDS patients in clinical setups. Additionally, the frequent exposure of HIV/AIDS patients to these antibiotics as a result of frequent infection related with reduced immunity as well as increased rate of hospitalization. On the other hand, all isolates

were susceptible for Nitrofurantoin. This is presumably due to Nitrofurantoin is not a commonly used antibiotics for empirical treatment of bacterial infection that preserves the potency of the drug. Hence, it is good if nitrofurantoin is used as an alternative treatment choice in treating resistant isolates.

Regarding to multidrug resistance, the overall MDR rate of all isolated *Enterobacteriaceae* in the current study was 92.9%. The highest prevalence of MDR might be related to different factors. Primarily, due to poor sanitation, inadequate health care services and poor access to drugs; the other factor is related with health care provider related factors in that the health care professionals sell the antimicrobials to patients or receive a kick back fee or other incentives by referring the patients to a particular pharmacy and even prescribe presumptively when there are no clinical indications. Additionally, patient related factors in that the patients believe and perceive that most infections respond to antibiotics and expect to be antibiotics by health care providers for those of non specific symptoms that may might not be even any particular infection. As these factors are highly pronounced in HIV/AIDS patients, the rate of MDR might be more pronounced in HIV /AIDS patients (10).

The prevalence of MDR (92.9%) among *Enterobacteriaceae* uropathogens was also assessed in this study. The finding of this study is comparable with reports in Gondar (93.5%) (74), Bahirdar (92.2%) (72) and Hawasa (90.8%) (69). But, it is higher than figures reported in Gondar (87.4%) (73), Dessie (74.9%) (71). However, it was lower than reports from Jimma (100%) (70). It is also indicated that the prevalence of MDR in the current study is higher than other reports like Mozambique (88.2%) in Africa (68) and other countries, such as USA (19.1%) (66), Nepal (41.1%) (67). The variation in prevalence of MDR *Enterobacteriaceae* isolates could be due to increase trend of MDR strains with time, difference in study period and study population. Moreover, high prevalence of MDR was observed among ESBL-PE (100%) than non producing isolates (90.9%) as demonstrated in various reports (9,62) which might be associated with unique property of the large ESBL plasmid, which is capable of incorporating and subsequently coding for resistant determinants to non beta-lactam antimicrobial agents in addition to beta lactam drugs.

6. LIMITATIONS OF THE STUDY

The current study has not sought molecular patterns of ESBL-PE isolates to demonstrate which molecular type was prevalent.

7. CONCLUSION

An alarming prevalence of ESBL-PE isolates were observed among HIV/AIDS patients and the *E. coli* and *K. pneumonia* were the most common ESBL-PE isolates. All ESBL producing isolates were MDR for all tested antimicrobial agents. Antibiotic resistance rates in ESBL producing isolates were comparatively higher than other ESBL non producing isolates.

8. RECOMMENDATION

Therefore, the current study indicates that the prevalence of ESBL producing isolates are alarmingly increasing that demands for the efforts should be made to reduce patient hospital stay and maximize rational use of drugs. Additional and vigorous investigation especially on ESBL producing isolates should be encouraged. There should also be a trend of routine screening of clinical isolates for ESBL production in clinical settings. It is also recommended that infection control practices should be strictly implemented to avoid the spread of resistant strain especially ESBL-PE. Most importantly, local antimicrobial susceptibility testing has to be promoted for the best management of HIV/AIDS patients.

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ANNEXES

Annex I: Information sheet and consent form

Study Title: Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV patients: Prevalence, antimicrobial susceptibility pattern and associated risk factors in University of Gondar Hospital, North west Ethiopia

Name of the Sponsor: Amhara Regional Health Bureau

Name of the Organization: School of Biomedical and Laboratory Sciences, Gondar College of Medicine and Health Sciences, University of Gondar.

1. Purpose of the Research Project: The aim of this study is to assess the prevalence of extended spectrum beta lactamase producing *Enterobacteriaceae* isolated from clinical samples among HIV patients attending at University of Gondar hospital, Gondar, North West Ethiopia. In line with the findings, it will provide a baseline information for health sector administrators and concerned bodies in planning and managing of drug resistance that allow to devise treatment and preventive measures such as a rational antibiotic use policy and plan a proper hospital infection control strategy to prevent the dissemination of these strains.

2. Procedure: In order to perform the indicated study at University of Gondar hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent**.** The required clinical sample will be collected by trained Laboratory technologists who are currently working in ART laboratory of the hospital. Then, you will be requested to give your consent to the sample collector. Socio-demographic, associated factors and clinical information will be collected from the study participants using structured questionnaires via interview technique; since you fulfill the criteria you are kindly requested to give the required sample and genuine answers to the prepared questionnaire.

3. Risk associated with the study: You will not be at any physical or psychological risk and no damage resulting from the research procedures but during collection of the blood you may feel some discomfort, this will not produce serious pain.

4. Benefits of the study: Based on the diagnosis result you will be treated accordingly. Moreover, this study will have a great value on preventive measures in hospitals and in the community. The results of this study have importance to treat the patients and to use as a baseline for effective treatment in the absences of laboratory investigation for empirical treatment of infections.

5. Compensation for participation: You will not receive any payment for your participation in this research study.

6. Confidentiality of your information- All information gathered from the study participant will remain confidential. Your participation in this study is strictly anonymous. Personal information will be treated confidentially and under no circumstances will it be transmitted to any person or organization. The results of this study will be evaluated and summarized, and a feedback of the results to the study participants will be given by principal investigator.

7. Right to Refusal or Withdraw: Your participation in the study is absolutely voluntary; you have full right to refuse from participating in this research. You can refuse to give sample and not to respond any or all the questionnaires and this will not affect you on using any kind of services from the hospital.

8. Person to Contacts: This research project will be reviewed and approved by Ethical review committee of School of Biomedical and laboratory Sciences, College of Medicine and Health Sciences, university of Gondar. If you want to know more information, you can contact the following individuals and you may ask at any time.

1. School of Biomedical and Laboratory Sciences, UGO

P.Box-196, Gondar, Ethiopia

2. Mr. Demeke Endalamaw, principal investigator

Mobile: +25193404064/ e-mail: demekeendalamaw@gmail.com

3. Mr Setegn Eshetie,(advisor), University of Gondar

Mobile: 0947050546/e-mail: wolet03.2004@gmail.com

4. Mr Anteneh Amsalu,(advisor), University of Gondar

Mobile: +251910047320 e-mail: ant.amsalu@gmail.com

Consent form

Serial No----------Name of health institution----------Card No---------- Date----------

I, the undersigned study participant have been well-informed about the objective of the study entitled "**Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV patients: Prevalence, antimicrobial susceptibility pattern and associated risk factors in University of Gondar Hospital, North west Ethiopia**"

Name of the Sponsor: Amhara Regional Health Bureau

I am also told that all the information obtained at any course of the study is to be kept confidential. Moreover, I have also been well-informed of my right to keep hold of, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care and hospital access.

I agreed voluntarily to provide the requested samples from me as well as my child.

Name and signature of study participant _____ Date_____

Name and signature of investigator _____ Date_____

Assent form for child

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to me in the study. I have received permission from my parents/guardian(s) to participate in the study and I have agreed to participate in the study. I know that as I can stop the study at any time.

_____ _____

Signature of Child Date

_____ _____

Signature Investigator Date

Annex II: Amharic Version Study Participant Information and Consent Form

የመረጃና የስምምነት ዉል ቅጽ

የጥናቱ ርእስ - እስከ 3ኛ ደረጃ ያሉትን የሴፋሎሰስፖሪን ዝርያ ፀረ-ባክቴሪያን የተቋቋሙ የባክቴሪያ ዝርያዎችን በጎንደር ዩኒቨርሲቲ ሆስፒታል በሚታከሙ የ ኤች ኤይ ቪ ሕመምተኞች በማንኛውም ናሙና ውስጥ መኖራቸውን መለየት፤ በምን ያህል መጠን እንዳሉ ማወቅ፤ በምን መድሃኒት ሊድኑ እንደሚችሉ እና ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ማወቅ፡፡

የጥናቱ ደጋፊ - የአማራ ብሔራዊ ክልላዊ መንግስት ጤና ቢሮ፡፡

የድርጅቱ ስም-ጎንደር ዩኒቨርሲቲ ህክምና ና ጤና ሳይንስ ኮሌጅ የላቦራቶሪ እና ባዮሜዲካል ትምህርት ቤት

1. **የጥናቱ ዓላማ**- እስከ 3ኛ ደረጃ ያሉትን የሴፋሎሰስፖሪን ዝርያ ፀረ-ባክቴሪያ የተቋቋሙ የባክቴሪያ ዝርያዎችን በጎንደር ዩኒቨርሲቲ ሆስፒታል በሚታከሙ ህመምተኞች በማንኛውም ናሙና ውስጥ መኖራቸውን መለየትና፤በምን ያህል መጠን እንደሚገኙ ማወቅ እና በምን መድሃኒት ሊድኑ እንደሚችሉ ማወቅ ነው፡፡አያይዞም ከጥናቱ የሚገኘው ውጤት ለጤና ተቋም አስተዳዳሪዎች ለሚመለከታቸው አካላት እና ለሕሙማን ለራሳቸው በዚህ ጆርም የሚከሰተውን ሕመምና ሞት ለመከላከል መሠረታዊ መረጃዎችን ይሰጣል፡፡
2. **የአሰራር ሂድት**- ይህን ጥናት በጎንደር ዩኒቨርሲቲ ሆስፒታል ለመስራት የጥናቱ ተሳታፊ እንዲሆኑ ተጋበዘዋል፡፡ ለመሳተፍ ፍቃደኛ ከሆኑ የጥናቱን ዓላማ መረዳትና ፍቃደኝነትዎን መግለፅ ይጠበቃል፡፡ለዚህ ጥናት የሚያስፈልገው ናሙና የሚሰበሰበው በናሙና አሰባሰብ ዙሪያ በቂ ስልጠና በወሰዱ ባለሙያወች ይሆናል፡፡እንዲሁም በተጨማሪ ማህበራዊ ነክ አጋላጭ ሁኔታዎች እና የጤንነት ሁኔታን የሚያሳዩ ጥያቄዎች ከያንዳንዱ የጥናቱ ተሳታፊዎች በመጠይቁ መሰረት ይሰበሰባል፡፡እርስዎም መስፈርቱን እስካሟሉ ድረስ የሚያስፈልገዉን ናሙና ለመስጠት እና ለተዘጋጀው ጥያቄ ትክክለኛ ምላሽ እንዲሰጡ በትህትና ይጠየቃሉ፡፡
3. **ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት** - በዚህ ጥናት ዝርዝር አሰራር ሂደት ውስጥ አካላዊ ወይም አእምሮአዊ ጉዳት አይኖርም፡፡ ነገር ግን ናሙናዉ በሚወሰድበት ጊዜ መጠነኛ የሆነ የህመም ስሜት ሊሰማዎት ይችላል፡፡ ቢሆንም ይህ የህመም ስሜት ምንም አይነት ችግር አያመጣብዎትም፡፡
4. **ጥቅሞች** - በምርመራ ውጤትዎ መሠረት ሕክምና ያገኛሉ በተጨማሪም ይህ ጥናት የመከላለል ስራ በሆስፒታል እና በህብረተሰቡ ዘንድ እንዲኖር ያግዛል፡፡ እንደዚሁም ደግሞ የላቦራቶሪ አገልግሎት በሌለበት ጤና ተቋም ትክክለኛ መድሃኒት ለመስጠት እንደ አመላካች ሆኖ ያገለግላል፡፡

5. **ለተሳትፎ የሚሰጥ ማካካሻ-** ምንም አይነት የካሳ ክፍያ የለውም
6. **የጥናቱ መረጃ ሚስጥራዊነት-** ሁሉም ከተሳታፊዎች የሚሰበሰቡ መረጃዎች በሚስጢር የሚያዙ እና የሚጠበቁ ይሆናሉ፡፡ በማንኛውም ምክንያት ተሳታፊዎች እነማን መሆናቸውን የሚያሳይ በመጠይቁ ይሁን በሌላ ነገር አይኖርም፡፡ የተሰበሰቡ መረጃዎች ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡ በተጨማሪም ውጤቱ የሚለካዉ ይሁን ተሰብስቦ የሚያዘው በዋና አጥኚ ነው፡፡
7. **የመዉጣት (የማቋረጥ) መብት** - በዚህ ጥናት ላይ መሳተፍዎ በሙሉ ፍቃደኝነት ላይ የተመሰረተ ነው ::ጥናቱን የማቋረጥ ሙሉ መብት አለዎት ፡፡ ናሙናም ሆነ ለመጠይቁ መልስ ያለመስጠት ከሆስፒታሉ የሚያገኙትን ማንኛውንም አገልግሎት አይገድብም ፡፡
8. **የሚያገኙዋቸው ሰዎች-** ይህ ጥናት በጎንደር ዩኒቨርሲቲ የስነምግባር ምርምር ኮሚቴና ሕክምናና ጤናሳይነንስ ኮሌጅ የላቦራቶሪ ባዮሜዲካል ትምህርት ክፍል ተዕልኮ የሚጸድቅ ይሆናል፡፡ጥያቄ ካለዎት ተጨማሪ መረጃ ከፈለጉ በማንኛዉም ጊዜ ከዚህ በታች የተጠቀሰዉን አድራሻ መጠቀም ይችላሉ፡፡

    1. ባዮ ሜዲካል እና ላቦራቶሪ ሳይንስ፡፡
       ፖ.ሳ ቁ-196, ጎንደር ኢትዮጵያ
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የስምምነት ዉል

የአዋቂዎች የስምምነት ማረጋገጫ ፊርማ

ተራ ቁጥር----------የሚታከምበት ጤና ተቋም----------የካርድ ቁጥር----------

ቀን----------

እኔ ከዚህ በታች ስሜ የተጠቀሰዉና የፈረምኩት የጥናቱ ተሳታፊ እስከ 3ኛ ደረጃ ያሉትን የሴፋሎሰስፖሪን ዝርያ ፀረ-ባክቴሪያን የተቋቋሙ የባክቴሪያ ዝርያዎችን በጎንደር ዩኒቨርሲቲ ሆስፒታል በሚታከሙ የ ኤች ኤይ ቪ ሕመምተኞች በማንኛውም ናሙና ውስጥ መኖራቸውን መለየት፤ በምን ያህል መጠን እንዳሉ ማወቅ፤ በምን መድሃኒት ሊድኑ እንደሚችሉ እና ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ለማሳወቅ የሚደረገውን ጥናት አላማና ጥቅም በሚገባ ተረድቻለሁ፡፡ ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍ በራሴ ፍቃድ የሚወሰን መሆኑም ተገልጾልኛል፡፡ በተጨማሪም ከጥናቱ ባልሳተፍም ሆነ አቋርጬ ብወጣ ከሆስፒታሉ በማገኘዉ የህክምና አገልግሎት ምንም አይነት ችግር እንደማይደርስብኝ ተነግሮኛል፡፡በመሆኑም ከእኔም ሆነ ከልጄ ናሙና መዉሰድ አስፈላጊ መሆኑን ስለተስማማሁበት ለመስጠት ሙሉ ፈቃደኛ መሆኔን በፊርማዬ እገልጻለሁ፡፡

የተሳታፊ ስምና ፊርማ _____ ቀን _____

የተመራማሪ ስምና ፊርማ _____ቀን _____

የልጆች የስምምነት ማረጋገጫ ፊርማ

እኔ የጥናቱ ተሳታፊ የጥናቱን ሂደት እና በጥናቱ ጊዜ ስለሚያጋጥሙኝ ነገሮች በሚገባ የተረዳሁና በጥናቱ ለመሳተፍ ከአሳዳጊዎቼ/ ከቤተሰቦቼ ፍቃድ አግንቻለሁ፡፡ ከጥናቱ በማንኛዉም ደረጃ እራሴን ማግለል እንደምችል ተረድቻለሁ እናም በጥናቱ ለመሳተፍ ተስማምቻለሁ፡፡

የተሳታፊዉ ልጅ ፊርማ----------ቀን----------

የዋና ተመራማሪ ፊርማ----------ቀን----------

Annex III: Questionnaire & Laboratory data collection form

Investigation of Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV patients: Prevalence, antimicrobial susceptibility pattern and associated risk factors in the University of Gondar Hospital, Gondar, North west Ethiopia from Jan 1-March 31, 2017

Annex III a: Questionnaire:

Code No	
Hospital Card No	
Date of sample collection	
Health Organization	

Section 1: Demographic information:

S. No	Question	Code	Response
Q101	Sex	0 = Male, 1 = Female	
Q102	Age	---------- Years	
Q103	Ethnicity	----------	
Q104	Religion	_____	
Q105	Residence	0 = Urban, 1 = Rural	
Q106	Occupation		
Q107	Education status	1 = No education, 2 = Primary schools, 3 = Secondary schools 4 = College/University	

Section 2: clinical information

S. No	Questions	Code	Response
Q201	Patient Type	0= outpatient, 1 = inpatients	
Q202	If admitted patient, admission ward: _____Date of admission: _____		
Q203	Admitted to this health care facility from	0 = home 1=other health facility	

Section 3: Risk factor for ESBL-PE: please tick box (es) that apply to this patient

S. No	Questions	Code	Response
Q301	Previous hospitalization for the past 12 months, If yes, for how long days/weeks?	0= No 1= Yes ----------	
Q302	ICU admission for the past 12 months	0 = No 1= Yes	
Q303	Previous UTI infection for the past 12 months	0 = No 1= Yes	
Q304	Previous antibiotic therapy? If yes, what type of drug have you been taking?	0 = No 1= Yes ----------	
Q305	Antibiotic use without prescription	0 = No 1= Yes	
Q306	Presence of urinary catheter	0 = No 1= Yes	
Q307	Mechanical ventilation	0 = No 1= Yes	
Q308	Surgery in the last 6 months	0 = N 1= Yes	
Q309	Chronic diseases (Diabetes, malignancy….)	0 = No 1= Yes	
Q310	Pregnancy	0 = No 1= Yes	
Q311	Use of contraceptive	0 = No 1= Yes	
Q312	History of haemodialysis	0 = No 1= Yes	
Q313	Use of Cotrimoxazole prophylaxis therapy	0 = No 1= Yes	
Q314	If yes, for Q 14, was completed?	0 = No 1= Yes	
Q315	ART status	0 = Not on ART 1= on ART	
Q316	WHO AIDS staging	1=Stage 1, 2=stage 2 3=Stage 3, 4=Stage 4	
Q317	CD_4 count? (Observe the patient chart)	----------	
Q318	None of the risk factors apply to this patient	0 = No 1= Yes	

Annex III b: Laboratory data collection form:

Code No	
Hospital Card No	
Date of sample collection	
Health Organization	

S. No	Question	Code	Response
1.	Enterobacteriaceae	1 =*E. coli*, 2 = *k. pneumonia* 3 = *Proteus spp*. 4 = *Enterobacter spp*. 5 = *Citrobacter spp*. 6 = other	
2.	ESBL-PE	0 = No 1 = Yes	

3. Antibiotic susceptibility pattern:

Antibiotic	Sensitivity Results (CLSI, 2017): Zone diameter breakpoints nearest to whole mm					
	Content	S	I	R	Reading (mm)	Interpretation
Cefuroxime	30µg					
Cefexime	30µg					
Cefotaxime	30µg					
Ceftazidime	30µg					
Ceftriaxone	30µg					
Cefpodoxime	30µg					
Cefepime	30µg					
Ciprofloxacin	5µg					
Cotrimoxazole	25µg					
Ampicillin	10µg					
Amikacin	10µg					
Amox-clav	30µg					
Nitrofurantoin	30 µg					

Annex IV: Amharic Version of Questionnaire

የአማርኛ መጠይቅ

እስከ 3ኛ ደረጃ ያሉትን የሴፋሎሰስፖሪን ዝርያ ፀረ-ባክቴሪያን የተቋቋሙ የባክቴሪያ ዝርያዎችን በጎንደር ዩኒቨርሲቲ ሆስፒታል በሚታከሙ የ ኤች ኤ ቪ ሕመምተኞች በማንኛውም ናሙና ውስጥ መኖራቸውን መለየት፤ በምን ያህል መጠን እንዳሉ ማወቅ፤ በምን መድሃኒት ሊድኑ እንደሚችሉ እና ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ለማወቅ የተዘጋጀ መጠይቅ፡፡

የኮድ ቁጥር	
የመታከሚያ ካርድ ቁጥር	
ናሙናዉ የተወሰደበተ ቀን	
የጤና ተቋሙ ስም	

ሀ. ማህበራዊ ና ኢኮኖማዊ መረጃ፤

ተ.ቁ	ጥያቄ	መለያ ምልክት	ምላሽ
1.	ፆታ	0 = ወንድ, 1 =ሴት	
2.	እድሜ	---------- አመታት	
3.	ብሄር	----------	
4.	ሐይማኖት	_____	
5.	መኖሪያ ቦታ	0 = ከተማ, 1 = ገጠር	
6.	ሥራ		
7.	የትምህርት ደረጃ	1= ያልተማረ 2= የመጀመሪያ ደረጃ 3= የሁለተኛ ደረጃ 4= የከፍተኛትምህርት	

ለ. ክሊኒካል መረጃዎች

ተ.ቁ	ጥያቄ	መለያ ምልክት	ምላሽ
1.	የታካሚዉ አይነት	0= ተመላላሽታካሚ,1= ተኝቶታካሚ	
2.	ተኝቶታካሚ ከሆነ የተኛበት ክፍል_____የተኛበት ቀን_____		
3.	ታካሚው ወደ ጤና ተቋሙ የመጣው	0= ከቤቱ 1= ከሌላ ጤና ተቋም	

ሐ. ለበሽታው መባባስ አጋላጭ ሁኔታዎች

ተ.ቁ	ጥያቄ	መለያ ምልክት	ምላሽ
1.	ላላፉት12 ወራት ሆስፒታል ተኝተው ያውቃሉ?	0= አይደለም 1= አዎ	
2.	ላላፉት12 ወራት በዕኑ ታመው ሆስፒታል ተኝተው ያውቃሉ?	0= አይደለም 1= አዎ	
3.	ላላፉት12 ወራት የ ሽንት ቱቦ ታመው ነበር?	0= አይደለም 1= አዎ	
4.	ፀረ-ባክቴሪያ መደሃኒት ወስደው ያውቃሉ? ስሙን ይጥቀሡ? ለምንያህልጊዜ?	0= አይደለም 1= አዎ	
5.	ከሃኪም ትዕዛዝ ዉጪ መድሃኒት የመግዛት ልምድ አለዎት?	0= አይደለም 1= አዎ	
6.	የሽንት ማሸኛ ቱቦ ተገጠሞለዎት ያውቃል?	0= አይደለም 1= አዎ	
7.	አየር መቅዘፊያ መሳሪያ ይጠቀማሉ?	0= አይደለም 1= አዎ	
8.	ላለፉት 6 ወራት ቀዶ ጥገና አድርገው ያውቃሉ?	0= አይደለም 1= አዎ	
9.	ስር የሠደደ ወይም ለረጅም ጊዜ የቆየ እንደ ስኳር ዓይነት ህመም አለብዎት?	0= አይደለም 1= አዎ	
10.	የእርግዝና ሁኔታ	0= የለም 1= አለ	
11.	የእርግዛና መከላከያ መድሃኒት ይጠቀማሉ?	0= አይደለም 1= አዎ	
12.	የኩላሊት እጥበት ዐካሂደው ያውቃሉ?	0= አይደለም 1= አዎ	
13.	ኮትሪሞክሳዞል የተባለውን መድሃኒት ለቅድመ መከላከያ ተጠቅመው ያውቃሉ?	0= አይደለም 1= አዎ	
14.	መልሥዎ አዎ ከሆነ የታዘዘልዎትን በሙሉ ጨረሰዋል?	0= አይደለም 1= አዎ	
15.	የ ኤ አር ቲ መድሃኒት ጀምረዋል?	0= አይደለም 1= አዎ	
16.	በአለም ጤና ድርጅት የበሽታው ደረጃ	1= ደረጃ 1, 2= ደረጃ 2 3= ደረጃ 3, 4= ደረጃ 4	
17.	ማንኛውንም አጋላጭ መንስኤዎች እርስዎ ላይ ተግባራዊ አይሆንም?	0= አይደለም 1= አዎ	

Annex V: Procedures

A. Urine Specimen collection

1. Instruct the patient to clean the urethral area thoroughly to prevent external bacteria from entering the specimen.

Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a clean-catch' specimen.

A. ***Female patients***: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

B. ***Male patients***: Wash the hands before collecting a specimen (middle of the urine flow). ***Note***: When a patient is in renal failure or a young child, it may not be possible to obtain more than a few milliliters of urine

2. Give the patient a sterile, dry, wide-necked, leak proof container and request to bring 10–20 ml urine specimen.
3. Let the patient void into the container.
4. Label the specimen container with patient identifying information, and send to the lab immediately. A delay in examining the specimen may cause a false result.
5. Wash your hands and instruct the patient to wash his hand as well

B. Culture media preparation

1. Read the manufacturer's instructions on the bottle of dehydrated agar media of interest.
2. Measure the appropriate amount of dehydrated powder media and distilled water using an electronic balance on clean dry paper and measuring cylinder respectively.
3. Drop a measured amount of distilled water in an Erlymeyer flask and add the measured amount of powder.
4. Close the flask with cotton foiled with aluminum foil
5. Heat the flask on a hot plate to dissolve the mixture until it vapors
6. When the agar mixture is completely dissolved, remove the flask from the hot plate
7. Autoclave the agar mixture for 15 min at 120^{o}c. Do not autoclave the preparation if indicated in the manufacturer's instruction.

8. Remove the flask of sterilized agar from autoclave and allow it to cool to about 50°C.
9. Quickly pour the melted, sterile agar into a series of petri dishes until about one-third of the dish is filled and wait until it solidifies by partially covering the dishes.
10. Invert the petri dishes if the agar is solidified to avoid the moisture from accumulating on the agar surfaces that has been condensed from the vapor.
11. Incubate one petridish from each batch of preparation in the incubator from for at least 24 hours to check sterility of preparation before use.

C. Gram stain procedures

1 Fix the dried smear as explained in subunit 7.3.2. *Note*: When the smear is for the detection of gonococci or meningococci, it should be fixed with methanol for 2 minutes (avoids damaging pus cells).

2 Cover the fixed smear with crystal violet stain for 30–60 seconds.

3 Rapidly wash off the stain with clean water. *Note*: When the tap water is not clean, use filtered water or clean boiled rainwater.

4 Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.

5 Wash off the iodine with clean water.

6 Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water. *Caution*: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.

7 Cover the smear with neutral red stain for 2 minutes.

8 Wash off the stain with clean water.

9 Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry.

10 Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

D. Biochemical testing procedures

Identification of Gram negative bacteria: will be based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, mannitol, urea agar and motility medium.
3. Incubate at 35-37 ^{O}c for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the six biochemical tests and their motility characteristics by comparing with the identification chart.

E. Antimicrobial susceptibility testing

1. Prepare a suspension of the test organism by emulsifying several colonies of the organism in a small volume of nutrient broth until it matches with a turbidity of 0.5 McFarland standard
2. Take a sample from the suspension with a sterile swab (squeeze the swab against the side of the test tube to remove the excess fluid).
3. Spread the inoculums evenly over Muller-Hinton agar plate with the swab
4. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
5. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
6. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
7. Interpret the reaction of the test organism to each antibiotic used as sensitive, intermediate, or resistance as per the standard

   Sensitive– zone of inhibition is wider or equal to the CLSI recommended zone size for each isolated strain

   Intermediate– If the zone of inhibition is within the intermediate range of CLSI guideline

Annex VI: Dummy Tables

Table 1: Socio-demographic characteristics of HIV patients at the University of Gondar Hospital, 2017

Variables		Frequency	Percent	P value
Age in year	<30			
	30-44			
	>44			
Sex	Male			
	Female			
Education	Primary and below			
	Secondary			
	Collage/University			
Residence	Urban			
	Rural			
Occupation	Farmer			
	Daily labourer			
	Merchant			
	House wife			

Table 2: ESBL production among the Gram negative isolates on HIV patients at the University of Gondar Hospital, 2017

Bacterial spcies	Number of ESBL producer(%)	Number of ESBL non producer(%)

Table 3: Antibiotic susceptibility pattern of ESBL producing isolates on HIV patients at the University of Gondar Hospital, 2017

Bacterial spcies	Interpretatin	Antibiotics															
		Cefotaxime	Cefpodoxime	Ceftriaxone	Ceftazidime	Cefepime	Gentamycine	Chlorampheni	Cipro	Nitrofur	Cotrimoxazol	Norfloxacilin	Tetracycline	Ampicilin	Amox-Clave	Naldixic acid	Total (%)
	S																
	I																
	R																
	S																
	I																
	R																
	S																
	I																
	R																

Key: S=Sensitive R=Resistance
I =Intermediate

Table 4: Association of ESBL infection and risk factors on HIV patients at the University of Gondar Hospital, 2017

Risk factors		**ESBL-PE**		**COR**	**AOR(95% CI)**	**p value**
		Yes	**No**			
Previous antibiotic use	Yes					
	No					
Hospitalization	Yes					
	No					
Invasive procedure	Yes					
	No					
Intake of self prescribed antibiotics	Yes					
	No					
Oral contraceptive intake	Yes					
	No					
Pregnancy	Yes					
	No					
CD_4 count	<500					
	>500					
Previous history of UTI	Yes					
	No					
Cotrimoxazole prophylaxis treatment	Yes					
	No					
Adherence of cotrimoxazole	Complete					
	Incomplete					
ART treatment	Yes					
	No					
WHO AIDS staging	Stage I					
	Stage II					
	Stage III					
	Stage IV					

Declaration

The research work in this thesis entitled **"Extended spectrum beta lactamases producing *Enterobacteriaceae* among HIV/AIDS patients: Prevalence and antimicrobial susceptibility pattern at the University of Gondar Hospital, North West Ethiopia"** was carried out by me under the supervision of **Mr. Setegn Eshetie** and **Mr. Anteneh Amsalu** at the University of Gondar, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Medical Microbiology, for the award of MSc Degree in Medical Microbiology. I declare that this work is original and has not been submitted to any other University or institution.

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Place: Gondar, 2017 Sign: _____ Date: 30/06/2017

Advisors	Sign	Date
1. Setegn Eshetie	_____	30/06/2017
2. Anteneh Amsalu	_____	30/06/2017

Examiners	Sign	Date
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